Echocardiographic Assessment of Right Ventricular Systolic Function in Conscious Healthy Dogs

THESIS

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Abstract

There is accumulating evidence demonstrating the important role of the echocardiographic assessment of right ventricular (RV) systolic function in people affected with a variety of cardiovascular diseases. In dogs, the echocardiographic assessment of RV function is underutilized and reference values and validation studies in normal dogs are sparse. The purpose of this thesis was to study several echocardiographic indices of RV systolic function in a relatively large sample of conscious, healthy dogs (n = 80), generate reliable reference values, and validate these indices in a clinically relevant manner. Chapter 2 shows the feasibility of measuring tricuspid annular plane systolic excursion (TAPSE), percent fractional area change (FAC), pulsed wave tissue Doppler imaging-derived peak systolic myocardial velocity of the lateral tricuspid annulus (S’), and speckle tracking echocardiography-derived global longitudinal RV free wall strain and strain rate. These variables were demonstrated to be repeatable indices of RV systolic function in conscious healthy dogs. Body weight-specific reference values were calculated for these estimates of RV systolic function. Chapter 3 demonstrates that these same 5 indices of RV function can track expected positive and negative inotropic changes in RV function compared to baseline following single oral doses of pimobendan and atenolol, respectively. The results of this thesis support the use of these echocardiographic indices for the clinical assessment of RV systolic function and calls for further study of these indices in dogs affected with cardiovascular disease.
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Chapter 1:
General Introduction and Review of the Literature

General Introduction

In both human and veterinary cardiology, the assessment of right ventricular (RV) structure and function has largely been overshadowed by the left ventricle (LV). The right ventricle was once considered a passive conduit, perhaps largely owing its neglect to the Fontan procedure, a palliative procedure for people affected with complex congenital heart disease in which the right atrium is directly connected to the pulmonary artery (PA) thus bypassing the RV.1 Recently, however, a growing body of literature on the study of RV function in people has unveiled its pivotal role and prognostic value in numerous cardiovascular disease states.2-6 Accordingly, further study of RV structure and function has been encouraged in human6 and veterinary medicine.7 The contents of this thesis offer a starting point for the study of RV function in dogs affected with cardiovascular disease by establishing reference values and validating echocardiographic indices of RV systolic function from a large sample of healthy dogs.

In the remainder of this chapter some of the current knowledge of RV development, anatomy, and physiology is reviewed. The currently used clinical indices of RV function along with their impact are also discussed. In chapter 2, a study determining the feasibility, repeatability and reference values for 6 echocardiographic indices of RV
function in normal dogs is presented. Chapter 3 attempts to validate the same indices in a clinically relevant manner by testing the indices’ ability to track expected changes in RV function. Lastly, in chapter 4, concluding remarks and future directions are presented.

**Review of the Literature**

*Right Ventricular Embryology and Anatomy*

The RV outflow tract and the apical portion of the RV develop from a group of myocardial precursor cells located anterior to the early heart tube, called the anterior heart field, whereas the atria, LV and remainder of the RV (inlet portion) stem from the primitive heart tube (primary heart field). The separate embryological origins of the ventricles are thought to explain why RV myocytes respond differently from LV myocytes to abnormal hemodynamic conditions such as pressure overload. Normally, the dextroventricular loop positions the anatomic RV to the right and the anatomic LV to the left of the animal’s midline. The spiraling decent of the aortopulmonary septum toward the LV with concurrent fusion of the conotruncal ridges results in formation and septation of the outflow tracts. A “crisscross” orientation of the right and left ventricular outflow tracts consequently manifests with the RV outflow tract located anterior (cranial) to the LV outflow tract. For the LV, subaortic conal absorption occurs, thus explaining the lack of an infundibular component and the distinctive mitral-aortic continuity of the LV.

After birth, the RV undergoes significant changes as it is functionally the dominant chamber in the embryo and developing fetus. In utero, the RV pumps only 15-
20% of the cardiac output into the high-resistance fetal pulmonary circulation and the remainder is pumped into the lower-resistance fetal systemic circulation through a large nonrestrictive ductus arteriosus and the foramen ovale.\textsuperscript{12} RV and LV free wall thickness and force development are equal throughout fetal life with the interventricular septum flat and midline throughout the cardiac cycle.\textsuperscript{13} After birth and throughout infancy, pulmonary vascular resistance decreases and there is gradual reduction in thickness of the RV relative to the interventricular septum and LV. This relative change in RV thickness is not considered regression of RV hypertrophy per se; it is more precisely explained by a discordance of the rate of growth of each ventricle with age, with the left ventricle exhibiting a much more rapid growth rate than the right.\textsuperscript{14,15}

In the normal dog, the right ventricle is the most cranial cardiac chamber that is located between the annulus of the tricuspid valve and the pulmonary valve. The RV is divided into three components: 1) the inlet, consisting of the tricuspid valve, chordae tendinae, and papillary muscles; 2) the trabeculated apical myocardium; and 3) the infundibulum, or conus, consisting of the RV outflow tract. An alternate more traditional division of the RV commonly encountered throughout the literature is that of the sinus (body of the RV) and conus (outflow tract).\textsuperscript{16} When compared to the ellipsoid shape of the LV, the RV three-dimensional (3D) shape is more complex. In the longitudinal plane it appears triangular-shaped and in the cross-sectional plane it is crescent-shaped. Despite pumping equal volumes, the mass of the human RV is approximately one-sixth that of the LV.\textsuperscript{17}
The myofiber orientation of the RV consists of superficial layers that are arranged circumferentially in a direction that is parallel to the atrioventricular groove but become arranged in an oblique manner as they advance toward the apex and the LV.\textsuperscript{11} In contrast, the deep RV myofiber layers are arranged longitudinally, aligned from base to apex. Compared to the RV, the myofiber orientation and contractile motion of the LV are more complex. The LV contains superficial myofiber layers that are arranged obliquely, subendocardial layers that are arranged longitudinally, and circumferentially-oriented fibers in between. This results in the more complex LV movement consisting of torsion, rotation, and thickening.\textsuperscript{11} The interventricular septum is generally considered part of the LV, but it does contain some longitudinal fibers that belong to the RV.\textsuperscript{18} The right and left ventricular myofiber continuity, along with the interventricular septum and pericardium, contribute to the interaction between the two ventricles throughout the cardiac cycle – so-called ventricular interdependence.

\textit{Right Ventricular Physiology}

The RV’s primary function is to receive systemic venous return and pump it into the relatively low-resistance pulmonary arteries. It does so by contracting in a sequential pattern starting with the inlet and trabeculated myocardium and ending with the infundibulum.\textsuperscript{11} Contraction of the RV occurs by three separate mechanisms: 1) movement of the free wall toward the septum, producing a bellows effect; 2) contraction of the longitudinal fibers, thus pulling the tricuspid valve annulus toward the apex; and 3) traction of the free wall at the points of attachment secondary to LV contraction.\textsuperscript{4,11} Using
tagged cardiac magnetic resonance imaging (CMR), shortening of the RV has been shown to be greater in the longitudinal plane than in the radial plane and this is likely due to the higher surface-to-volume ratio of the RV, thus requiring a lesser inward motion to eject the same stroke volume as compared to the LV.\textsuperscript{19} The characteristics of RV contraction are highly dependent on its loading conditions. RV output approximates that of the LV, but is achieved with a myocardial energy cost about one-fifth that of the LV due largely to the low resistance pulmonary circulation into which the RV ejects.\textsuperscript{18}

Under normal conditions, the RV is coupled with a low-impedance, highly distensible pulmonary vascular system. Right ventricular pressure tracings show an early peaking and rapidly declining pressure, contrasting to the more rounded contour of LV pressure traces.\textsuperscript{16} Right ventricular isovolumetric contraction time is shorter than the LV’s because RV systolic pressure rapidly exceeds the low PA diastolic pressure. Echocardiographically, the Doppler flow profile at the level of the PA exhibits a more rounded, symmetrical appearance with peak velocity noted mid-way through RV ejection, as compared to the Doppler flow profile at the level of the aortic valve in which the velocity peaks in the first one-third of LV ejection. The longer acceleration time of the PA profile is most likely due to the reduced vascular resistance of the pulmonary circulation compared to systemic circulation. A so-called “hangout interval” is also unique to the RV. First characterized by Shaver and colleagues in 1974,\textsuperscript{20} the hangout interval is the time difference between the pulmonary arterial dichrotic notch (representing pulmonary valve closure) and simultaneous RV pressure measurement. In the normal RV, pulmonary valve closure occurs well after the onset of RV pressure
decline indicating that end-systolic forward flow may continue in the presence of a negative ventricular-arterial pressure gradient.\textsuperscript{21} This is most likely explained by the momentum of blood in the RV outflow tract.\textsuperscript{21} Increasing RV afterload has been shown to shorten the hangout interval.\textsuperscript{21}

The pressure-volume relationship of the RV exhibits a characteristic triangular or trapezoidal shape with ill-defined isovolumetric periods, especially the isovolumetric relaxation period.\textsuperscript{22} Interestingly, studies describing the pressure-volume relationship of the ventricles show the LV exhibits a contour nearly identical to the RV when the anatomic LV is matched with the pulmonary circulation (e.g., in the setting of congenitally corrected transposition) and the RV pressure-volume loop exhibits a change in contour similar to the LV following a chronic gradual pressure overload.\textsuperscript{23} These studies illustrate the afterload-dependency of RV contractile performance. The RV has also been shown to be more sensitive to changes in afterload compared to the LV,\textsuperscript{24} which likely explains its relative intolerance to pressure overloads and its increased propensity to fail in this setting compared to the LV.\textsuperscript{4}

Ventricular interdependence is important to discuss in the setting of RV physiology and its concept is first credited to Bernheim in 1910.\textsuperscript{16} It refers to the concept that the size, shape, and compliance of one ventricle may affect the size, shape, and pressure-volume relationship of the other through direct mechanical interaction. Consequently, it is now well-known that normal RV contractile function is dependent on that of the LV. In the setting of RV dysfunction an estimated 20–40\% of RV function is derived from the LV, which results largely from the movement of the interventricular
Although constantly present, ventricular interdependence becomes more apparent during changes in loading conditions, such as those seen with respiration and its associated changes in RV preload due to intrapleural pressure changes contributing to changes in venous return to the right heart. As previously mentioned, the ventricles are functionally linked due to common superficial myofibers, the pericardium, and the interventricular septum. Systolic ventricular interdependence is primarily regulated by the interventricular septum, whereas diastolic interdependence is regulated by a combination of the pericardium and interventricular septum.

Overall, regulation of RV function is similar to that of LV function. Mechanisms that acutely regulate RV function include heart rate, the Frank-Starling mechanism and the autonomic nervous system. Interestingly, the autonomic nervous system has been shown to have a differential effect on the inflow and outflow regions of the RV. Sympathetic or inotropic stimulation has been demonstrated to show a more pronounced effect on the outflow region compared to the inflow portion of the RV.

Clinical Assessment of Right Ventricular Function

Accurate noninvasive assessment of RV function is challenging, particularly when compared to the LV. This is largely because of the RV’s complex shape, limited endocardial border detection, and its load-dependency. Like the LV and despite its flaws, the clinical index of RV contractility is ejection fraction (EF). In humans, CMR is considered the noninvasive “gold standard” technique to quantify RV volumes and determine EF. CMR has been utilized to assess RV function in a small set of Boxer
dogs with arrhythmogenic RV cardiomyopathy (ARVC) and, compared to controls, EF was lower in the ARVC group.\textsuperscript{30} Radionuclide ventriculography and computed tomography (CT) are also noninvasive clinical means of quantifying RV volumes in people. Radionuclide ventriculography has been studied in dogs with experimentally induced heart failure and results indicate that it may provide valuable adjunct information regarding the noninvasive assessment of RV function.\textsuperscript{31}

Echocardiography remains the ideal screening modality when RV dysfunction is suspected, as it is inexpensive, safe, widely available, and does not require general anesthesia. In people, numerous echocardiographic indices of RV function have been validated against invasive or noninvasive “gold standards”\textsuperscript{1,18,32-34} and recently guidelines and reference values have been established.\textsuperscript{34} This is in contrast to dogs where the echocardiographic assessment of RV function is underdeveloped, validation studies are absent, and reference values are scarce. Attractive echocardiographic indices of RV systolic function based on human\textsuperscript{18,32-34} and some veterinary studies\textsuperscript{35-40} include, the inherently simple M-mode-derived tricuspid annular plane systolic excursion (TAPSE) index, the 2D surrogate of RV EF – percent fractional area change (FAC), pulsed wave tissue Doppler imaging (TDI)-derived RV myocardial systolic velocity of the lateral tricuspid valve annulus (S’), and the relatively new speckle-tracking echocardiography (STE)-derived global longitudinal strain and strain rate indices. These indices are discussed in further detail throughout Chapters 2 and 3.

In addition to the 5 aforementioned indices of RV systolic function, other relatively well-studied echocardiographic indices in human patients include the RV
myocardial performance (Tei) index, TDI-derived isovolumic acceleration (IVA) index, and 3D echocardiography-derived RV EF. The RV myocardial performance index is attractive because it is a geometry-independent index of global RV function. It consists of the ratio of isovolumic time intervals to ventricular ejection time. There is a relatively large body of human literature on this index demonstrating that it correlates well with gold standards and reference values exist. This index has been studied in a relatively large sample of normal dogs, dogs with tricuspid valve regurgitation, and in Boxer dogs with ARVC. Disadvantages of this index include the necessity for two separate Doppler traces (from RV inflow and outflow) with ideally identical heart rates and the fact that systolic and diastolic dysfunction cannot be differentiated. The IVA index is calculated as the difference between baseline and peak velocity during isovolumic contraction divided by their time interval. Despite validation of this index in people and the fact that it is less load-dependent than other echocardiographic indices of RV systolic function, its routine use has not been recommended in people as reference values are lacking. 3D echocardiography-derived RV EF is the latest technology available for echocardiographic RV function assessment. Despite its time-consuming nature, this index may prove advantageous for clinical use, as promising validation studies and prognostic data are accruing in people. IVA and 3D echocardiography-derived EF have not been studied in a clinical setting for veterinary species.

Compared to LV diastology, the noninvasive study of RV diastolic function is relatively unexplored. It is particularly difficult to study, given the fluctuations of RV preload secondary to normal respiration, in addition to its phasic nature. To overcome
this, people are instructed to hold their breath usually at end expiration during the echocardiographic assessment in order to more accurately quantify RV diastolic function, an obvious difficulty in veterinary patients. One practical recommendation for the assessment of RV diastolic function, in addition to merely assessing right atrial size, is to determine if RV “restrictive physiology” is present. This consists of examining a PA Doppler tracing and searching for end-diastolic antegrade flow in the PA, suggesting RV end-diastolic pressure is higher than PA diastolic pressure. This is seemingly feasible and adaptable to veterinary species and may ultimately provide helpful clinical information.

Clinical Importance of Right Ventricular Function

The importance of the quantitative assessment of RV function with regard to clinical status and outcome is becoming increasingly apparent in people affected with various types of cardiovascular disease. Specifically, functional assessment of the RV plays an important role regarding therapeutic decisions and prognosis in congenital heart disease such as RV outflow tract obstructions, tricuspid valve malformations, atrial septal defects and other complex congenital heart diseases. A recent study in people affected with ARVC revealed that TAPSE and FAC are strong predictors of adverse outcomes and the authors advocated for the use of echocardiography for risk stratification in this disease. Numerous studies have now documented that STE-derived strain imaging of the RV also appears to be of value in the early detection of ARVC. In people affected with pulmonary hypertension, the clinical and prognostic value of several echocardiographic indices of RV systolic function has been well-documented including,
TAPSE, FAC, S', the RV myocardial performance index, and STE-derived strain and strain rate. In addition to right heart specific diseases, the echocardiographic assessment of RV systolic function has also been shown to aid in the clinical decision-making process, and to provide prognostic data in people affected with diseases that primarily affect the left heart, including valvular heart disease, hypertrophic cardiomyopathy, and idiopathic dilated cardiomyopathy, often independent of pulmonary hypertension status. The diagnostic value of TAPSE, spectral Doppler-derived systolic time intervals, and TDI of the RV have also been demonstrated in dogs affected with pulmonary hypertension.

Purpose and Hypothesis

Based on the information presented, it is clear that numerous echocardiographic indices of RV systolic function can track “gold standard” RV function measurements in humans. Importantly, several of these indices including, TAPSE, FAC, S’, strain and strain rate have been shown to provide valuable information regarding diagnosis, treatment, risk assessment, and outcome in people affected with cardiovascular diseases that similarly affect the canine population. Therefore, optimization of the echocardiographic assessment of RV systolic function in dogs is warranted because of its potential to guide diagnosis, therapy and prognosis in dogs affected with cardiovascular disease. However, studies attempting to validate these indices, in addition to reference values derived from a large population of healthy dogs, are essential prior to wide-spread clinical use.
The general objective of this thesis was to comprehensively study echocardiographic indices of RV systolic function: TAPSE, FAC, S’, global RV free wall strain and strain rate in a large sample of conscious healthy dogs. The specific aims were as follows: 1) to determine the feasibility, repeatability, interobserver variability and intraobserver variability of the RV function indices; 2) to determine the effect of age, gender, heart rate, and body weight on these RV function indices in order to generate reproducible, clinically-applicable reference values; and 3) to determine the ability of these echocardiographic indices to track changes in RV function following a single oral dose of the inodilator pimobendan and beta blocker atenolol when compared to baseline.

We hypothesized that: 1) the echocardiographic indices of RV systolic function are feasible in healthy dogs and carry acceptable repeatability, interobserver variability, and intraobserver variability; and 2) the proposed RV function indices can detect changes in RV systolic function following a single oral dose of pimobendan (increase in systolic function) and atenolol (decrease in systolic function) in conscious healthy dogs.
References


Chapter 2:

Echocardiographic Assessment of Right Ventricular Systolic Function in Conscious Healthy Dogs: Repeatability and Reference Values

Abstract

Objectives: To determine the feasibility, repeatability, intra- and interobserver variabilities, and reference values for pulmonary velocity time integral (VTI), tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), pulsed wave tissue Doppler imaging-derived systolic myocardial velocity of the lateral tricuspid annulus (S’), and speckle-tracking echocardiography-derived global longitudinal RV free wall strain and strain rate, and study the effects of age, gender, heart rate, and body weight on these echocardiographic indices.

Animals: 80 healthy adult dogs

Methods: All dogs underwent 2 echocardiographic studies between 3 and 20 days apart. Repeatability, intra-, and interobserver measurement variability were quantified by average coefficient of variation (CV). The relationships between each RV function index and age, gender, heart rate and weight were also determined by regression analysis.

Results: All RV systolic function indices could be acquired with adequate repeatability and intra- and interobserver variability. All average CVs were < 10%, except for interobserver variability for pulmonary VTI (11.3%). Given the higher degree of variability in VTI, it was not analyzed further. Regression analysis using allometric
scaling showed the following correlations to body weight: TAPSE exhibited a strong ($r^2 = 0.75$) positive correlation, S’ and strain rate exhibited a moderate ($r^2 = 0.31$ and 0.44, respectively) positive and negative correlation, respectively, and FAC and strain exhibited a weak ($r^2 = 0.22$ and 0.14, respectively) negative correlation. Strain rate and FAC exhibited a moderate ($r^2 = 0.35$ and 0.31, respectively) positive correlation with heart rate. Gender had no measurable effect on any of the RV function indices. Weight-based reference values for these 5 RV systolic function indices were determined.

Conclusions: The reported indices of RV function are feasible, repeatable and warrant further study in dogs with cardiovascular disease.

**Introduction**

The clinical assessment of right ventricular (RV) function in veterinary medicine is underdeveloped and has traditionally relied on qualitative opinion or signs of right-sided congestive heart failure to diagnose dysfunction. The qualitative assessment of RV structure and function is inaccurate and shows poor interobserver agreement in people. Quantitative indices of RV function are therefore desirable. The importance of the quantitative assessment of RV function with regard to clinical status and outcome is increasingly apparent in people affected with both cardiac and non-cardiac diseases. The right ventricle is affected by a number of disease processes, including pulmonary hypertension caused by lung or pulmonary vascular diseases, global or RV-specific cardiomyopathies (arrhythmogenic right ventricular cardiomyopathy), pericardial diseases, pulmonary or tricuspid valve malformations, some left-to-right shunts, and
complex congenital heart disease. In people there is compelling evidence that RV function provides prognostic data and aids in the clinical decision-making process not only in right heart-specific diseases\(^3\) but also left heart-specific diseases, including mitral and aortic valve disease\(^4-6\) and idiopathic dilated cardiomyopathy,\(^7-12\) often independent of pulmonary hypertension status. Thus, the study of the quantitative assessment of RV function in dogs is warranted and might provide clinically meaningful information that impacts quality and duration of life.

The assessment of RV structure and function is challenging, particularly when compared to the left ventricle, due to its relatively complex geometry. Specific challenges of assessing the RV include separate inflow and outflow regions, the limited definition of the endocardial surface due to prominent trabeculae, and the marked load-dependence of most indices of RV function.\(^13\) In veterinary medicine, echocardiography is the most practical method for assessment of RV structure and function, as it is noninvasive, readily available, relatively inexpensive, and does not require general anesthesia. In contrast to the veterinary situation, numerous echocardiographic indices of human RV function have published guidelines and reference values.\(^14\) Although each index has inherent advantages and disadvantages, nearly all of these human RV indices have been validated against a catheterization- or cardiac magnetic resonance-derived gold standard.\(^14-18\)

The velocity time integral (VTI) of the pulmonary outflow signal is directly proportional to stroke volume and can be considered a surrogate for cardiac output of the right ventricle when multiplied by heart rate, as this variable is an integration over time of
the flow out of the right heart. When multiplied by the cross-sectional area of the pulmonary valve annulus, stroke volume can be derived.\textsuperscript{19}

Tricuspid annular plane systolic excursion (TAPSE) is one of the simplest methods to assess RV longitudinal function and canine reference values have been recently published.\textsuperscript{20} Measurement of TAPSE involves determination of the apical displacement of the tricuspid valve annulus during systole from an M-mode recording.

Percent fractional area change (FAC) of the right ventricle represents a 2-dimensional (2D) surrogate of RV ejection fraction from an apical imaging plane and is calculated from planimetered measurements of the RV chamber at end-diastole and end-systole. The end-systolic area is subtracted from the end-diastolic area with the difference then divided by the end-diastolic area. In people, TAPSE and FAC are practical and routinely performed for the clinical assessment of patients with cardiac disease.\textsuperscript{16}

Tissue Doppler imaging (TDI)-derived systolic myocardial velocity of the lateral tricuspid annulus (S’) represents a region-specific index of RV longitudinal function. Pulsed wave TDI-derived RV myocardial velocity is appealing because it is easy to obtain and strongly correlates with an invasive gold standard in the dog.\textsuperscript{21}

Speckle-tracking echocardiography (STE) is a relatively new index of myocardial function and is based on quantifying tissue deformation (strain) or tissue deformation over time (strain rate) globally or in a region-specific manner. A large advantage of STE is that it is less angle-dependent, which is seemingly well-suited for the right ventricle given its complex geometry and trabeculated endocardium.
Aside from TAPSE, canine reference values for RV systolic function indices are currently lacking. Reference values and repeatability data from a large, healthy sample of the canine population are essential prior to wide-spread clinical application of echocardiographic indices in diseased dogs. As several echocardiographic indices of cardiac structure and function are known to be affected by age, gender and body size in humans\textsuperscript{22-29} and animals,\textsuperscript{30-34} these variables should be considered when establishing reference values. Scaling to body weight particularly warrants consideration for the most accurate assessment of reference values given the wide range of body sizes in dogs.

The first objective of this study was to determine the feasibility, repeatability and intra- and interobserver variability of 6 echocardiographic RV systolic function indices: VTI, TAPSE, FAC, S’, STE-derived global longitudinal RV free wall strain and strain rate. The second objective was to explore the effect of age, gender, heart rate, and body weight on those indices with acceptable variability in order to generate accurate, clinically-applicable reference values.

**Materials and Methods**

All procedures in this study were approved by the Institutional Animal Care and Use Committee and the Veterinary Medical Center Clinical Research and Teaching Advisory Committee at The Ohio State University. Written consent authorizing participation of dogs in the study was obtained from all dog owners.
**Study Subjects**

A convenience sample of eighty privately-owned healthy, mature dogs $\geq 8$ months of age and of varying breed and body weight ($n = 40 > 15$ kg; $n = 40 \leq 15$ kg) were recruited for this study. Dogs were determined to be healthy and without cardiac or respiratory disease based on medical history, routine physical examination, cardiovascular examination, and a thorough screening echocardiogram performed by the same investigator (LCV). Exclusion criteria for the study were as follows: 1) pathologic heart murmur, gallop sound, or (non-sinus) arrhythmia; 2) history of respiratory disease; 3) dogs taking medications known to affect the cardiovascular or respiratory system; 4) uncooperative temperament that might require sedation for an echocardiogram; 5) Boxer dogs and English bulldogs due to risk of undetected arrhythmogenic RV cardiomyopathy; and 6) an abnormality identified on a baseline 2-dimensional (2D), M-mode, and Doppler echocardiographic study. Due to the high prevalence of physiologic tricuspid and pulmonary valve regurgitation in dogs, the presence of tricuspid or pulmonary valve regurgitation based on color Doppler echocardiography (with normal valve morphology) that was silent to auscultation was not considered an exclusion criterion.

**Echocardiographic Examination**

Conventional and Doppler Echocardiography – All echocardiographic studies were performed by the same investigator (LCV) using a GE Vivid 7 Echocardiographic system with transducer selection (4, 7, or 10 MHz nominal frequency) matched to the size of the dog and presets for optimal canine imaging. Echocardiographic recordings
were made with a simultaneous electrocardiogram (ECG), and all raw data was captured digitally for off-line analysis at a digital workstation. Standard imaging planes were utilized with the dogs manually restrained in right and left lateral recumbency without the use of sedation. The pulmonary artery flow was recorded with pulsed-wave Doppler imaging from a standardized right parasternal short axis view using color Doppler imaging to guide placement of the sample volume (1-3 mm) centrally between the opened pulmonary valve leaflets.

**Echocardiographic Indices of Right Ventricular Systolic Function** – With the exception of pulmonary artery velocity time integral (VTI), all the indices of RV systolic function were acquired from the left apical 4-chamber view optimized for the right heart which usually involved transducer placement one intercostal space cranial to the standard left apical 4-chamber view. Care was taken to maximize the RV longitudinal dimension and to exclude the aortic valve region to avoid foreshortening of the RV. At least 10 cardiac cycles of each RV function index were acquired and stored for off-line analysis and the value recorded for each RV function index at each time point was determined from the average of 5 representative cardiac cycles. The heart rate value recorded represented the average heart rate of each of the 5 cardiac cycles used to determine the RV function index value. Care was taken to adjust instrumentation to optimize RV myocardial and endomyocardial border resolution and to record during periods of quiet/calm respiration.

The pulmonary artery VTI, representing a measure of mean distance throughout the flow period, was determined by tracing the outer border of the pulmonary artery flow
TAPSE measurement consisted of quantifying the maximal longitudinal displacement of the lateral tricuspid valve annulus toward the RV apex during systole and was generated from M-mode recordings with the cursor as parallel as possible to the majority of the RV free wall (Figure 2). In order to avoid underestimating TAPSE values, the anatomic M-mode technique was utilized on rare occasion (< 10% of recordings) on stored 2D cine loops when conventional M-mode recordings were judged to be suboptimal due to inadequate parallel alignment to the RV free wall or the inability to clearly identify the tricuspid annular motion on the M-mode recording.

TAPSE measurements were made at sweep speeds of at least 66 mm/s. Measurements of RV area for FAC determination were obtained by tracing the RV endocardial border at end-diastole (RVA_D) and end-systole (RVA_S) as shown in Figure 3. Right ventricular percent FAC was calculated using the formula: FAC = (RVA_D – RVA_S)/RVA_D x 100.

Pulsed-wave TDI velocities of longitudinal myocardial motion at the lateral tricuspid annulus were obtained to measure peak systolic annular velocity (S’) as shown in Figure 4. For accurate TDI imaging, the cursor was aligned as parallel as possible with the majority of the RV free wall, and frame rate was maximized (at least 125 frames/second). Measurements of S’ were made at sweep speeds of at least 66 mm/s.

Strain and strain rate measurements were made off-line with proprietary 2D speckle-tracking software utilizing the left ventricular 2Ch algorithm, as no defined RV STE algorithm was available at the time of this study. Because optimal frame rate for canine RV STE is currently unknown, three 2D RV cine loops each were acquired at maximum, at 1 setting less than maximum, and at 2 settings less than the maximum
frame rate. Event timing for pulmonary valve opening and closure was timed to the ECG via a continuous wave Doppler recording of pulmonary outflow. Only RV free wall longitudinal strain and strain rate were studied as longitudinal motion has been recognized to be the major contributor to RV contraction. The region of interest for STE was defined by manually tracing the RV free wall endocardial border from the level of the tricuspid valve annulus to the RV apex and manually adjusted to incorporate the entire RV free wall myocardial thickness. Individual RV segments were then visually analyzed to assure adequate myocardial tracking by the software and manually adjusted if necessary. In general, tracking was accepted if both visual inspection and software inspection (green color coding conformation) confirmed it was adequate (Figure 5). However, on rare occasion, when software approval was unobtainable it was manually overridden so long as visual inspection of myocardial tracking was considered adequate. The frame rate that provided the most accurate tissue tracking based on visual and software inspection was chosen for STE-derived strain and strain rate (generally > 80 frames/second). Strain and strain rate values were generated by the software for each of 3 myocardial segments (basilar, mid, and apical myocardium of the RV free wall) in addition to the global strain and strain rate from the entire RV (considered as a single segment and not a mean of the 3 segments). Only systolic global longitudinal systolic strain and strain rate values of the RV free wall were utilized in this study and were determined as the maximal (most negative) systolic point on the respective global strain or strain rate curve prior to pulmonary valve closure (Figures 6 and 7).
Repeatability, Intra- and Interobserver Measurement Variability

Day-to-day repeatability of each RV function index was evaluated by having each dog in the study population undergo 2 baseline echocardiographic studies performed by the same investigator (LCV) between 3 and 20 days apart. Intraobserver measurement variability was determined by having the same individual (LCV) measure identical RV function indices from 6 randomly selected echocardiographic studies on 3 separate occasions. Interobserver measurement variability was determined by having 2 trained individuals (LCV & BAS), blinded to each other’s measurements, measure identical RV function indices from 6 randomly selected echocardiographic studies. The 6 selected echocardiographic studies used to determine the intra- and interobserver measurement variability were determined by randomly choosing 3 echocardiographic studies from dogs weighing ≤ 15 kg and 3 echocardiographic studies from dogs weighting > 15 kg.

Statistical Analysis

All statistical analyses were performed using a commercial software packages. For the purpose of the reference values, values for each RV function index and heart rate were generated from the average of the 2 day-to-day repeatability data sets (pooled data) from each dog. Descriptive statistics (mean, median, standard deviation [SD], and 95% confidence intervals [CI] or interquartile range) were calculated for all baseline RV function indices. Normality testing for continuous data consisted of visual inspection of the probability plots and the D’Agostino-Pearson test. Data is reported as mean ± SD unless otherwise indicated. A value of P < 0.05 was considered statistically significant.
If each of the average CV for repeatability, inter- and intraobserver variability were considered adequate (< 10%), the RV function index was analyzed with various regression models. For all linear regression models, assumptions of linearity, homoscedasticity, and normality of the residuals were evaluated by inspection of the standardized residual plots and probability plots. Normality of the standardized residuals was also assessed with the D’Agostino-Pearson test. Standardized residual plots and Cook’s distances were used to identify possible outliers and influential data points on the model and if Cook’s distances of greater than 1 were encountered, the data point was excluded from further analysis. For the purpose of this study, the simplest mathematical model (i.e., the model with the fewest number of predictors) achieving the highest degree of statistical significance (i.e., the largest F-statistic) and adjusted $r^2$ value was considered the best model of fit. Constants for allometric modeling were derived using the logarithmic form of the allometric scaling equation: $\log(Y) = \log(a) + b \times \log(M)$, where $a$ is the proportionality constant, $b$ is the scaling exponent, $Y$ represents the RV function index, and $M$ represents body weight. Simple linear regression analysis yields the constant $b$, which is the slope of the regression line and the constant $a$, which is
the antilog (log^{-1}) of the y-intercept of the regression line. The 95% prediction intervals for the linear regression line of best fit were then used to calculate the recommended lower and upper reference values. An unpaired t-test (or Mann-Whitney U test) was used to test for differences between male and female dogs for each RV function index.

The average percent coefficient of variation (CV) was used to quantify day-to-day repeatability for the 2 time points, intraobserver measurement variability, and interobserver measurement variability where percent CV = (the standard deviation of the measurements / average of measurements) x 100.

Results

Study Subjects

The study sample consisted of 80 dogs with a mean age of 4.1 ± 2.5 years (range, 0.66 – 9 years) and a mean body weight of 18 ± 11 kg (range, 3.9 – 42.3 kg). Thirty-six dogs were castrated males and 44 were spayed females. Forty-one dogs were mixed breeds, 5 were Pugs, 4 dogs each were Boston Terriers and Labrador Retrievers, 4 dogs each were Golden Retrievers and Miniature Schnauzers, 2 dogs each were Cavalier King Charles Spaniels, Rat Terriers, Italian Greyhounds, Chihuahuas, Beagles, and German Shepherds. The other breeds (Border Collie, Wheaton Terrier, Bloodhound, Miniature Pinscher, Greyhound, Pembroke Welsh Corgi, English Setter, Toy Poodle, Pomeranian, and Papillon) were each represented once.
Repeatability, Intra- and Interobserver Measurement Variability

Each RV function index could be measured in all dogs. Descriptive statistics for each of the RV function indices from the baseline echocardiographic studies are summarized in Table 1.

With the exception of pulmonary VTI interobserver measurement variability, all average CV for repeatability, intra- and interobserver measurement variability of all RV function indices were considered adequate at <10% (Table 2). Given the variability in VTI measurement, it was not considered further in the analysis.

RV Function Indices in Conscious Healthy Dogs

Multiple linear regression analyses were performed on all indices except VTI and revealed that each RV function index was significantly (all P ≤ 0.023) correlated with body weight, whereas FAC and strain rate exhibited a significant (both P ≤ 0.001) moderate positive correlation with heart rate. Age did not significantly contribute to the model for any RV function index. The zero-order and part (semi-partial) correlation coefficients are presented in Table 3. The part (semi-partial) correlation coefficients, determining the unique variation each predictor shares with the dependent variable (while controlling for the effects of the other predictors), were considered the most meaningful estimates for prediction of the RV function index from the independent variables (predictors).

Allometric (power) regression exhibited the best model fit compared to simple linear, second-order polynomial and third-order polynomial regression models for all RV
function indices and body weight. Right ventricular function indices and body weight were significantly correlated (all $P \leq 0.001$) and the results of the allometric scaling model for each RV function index are summarized in Table 4. Correlation was highest for TAPSE ($r^2 = 0.749$), moderate for $S'$ and strain rate ($r^2 = 0.306$ and 0.440, respectively), and relatively weak for FAC and strain ($r^2 = 0.216$ and 0.140, respectively). Table 5 shows the suggested reference values for each RV function index for dogs of different body weights. These values represent the 95% prediction intervals for the regression line of best fit derived from the allometric equation.

Gender did not have a statistically significant effect on any of the RV function indices.

**Discussion**

The results of this study showed that TAPSE, FAC, $S'$, strain, and strain rate were feasible, highly reproducible and had intra- and interobserver variability within clinically acceptable limits. While pulmonary VTI was feasible and repeatable, it exhibited interobserver variability outside the preferred range and was therefore not analyzed further. This study revealed that the other 5 RV function indices were significantly correlated to body weight with TAPSE exhibiting a strong positive correlation, $S'$ and strain rate exhibiting a moderate correlation (positive and negative, respectively), and FAC and strain exhibiting a weak negative correlation to body weight. Strain rate and FAC also exhibited a moderate positive correlation with heart rate, which the clinician should be mindful of particularly when heart rates significantly deviate from the norm. Lastly, body
weight-specific reference values from a relatively large sample of conscious healthy dogs
are presented so that an accurate quantitative assessment of RV systolic function can be
performed.

Despite encouragement, the clinical assessment of RV structure and function
has remained relatively underdeveloped in veterinary medicine. Previous studies on the
assessment of RV systolic function in dogs have focused on comparing diseased (natural
or experimental) dogs to a relatively small number of controls or have compared RV
function to LV function. While these studies provide valuable information, it is
difficult to extrapolate these indices to wide-spread clinical use without establishing
intervals that differentiate diseased dogs from healthy dogs.

Others have examined echocardiographic indices of RV function in relatively
large samples of healthy dogs, including color TDI-derived RV myocardial velocities,
TAPSE, RV systolic time intervals, RV shortening fraction, and a RV myocardial
(Tei) index. However, with the exception of TAPSE, reference values were either not
reported or were reported as central tendencies for estimating the mean (e.g., confidence
intervals of the mean or standard deviation) and not as central tendencies for estimating
values for the population. Some of these studies have also documented a significant effect
of body weight on the RV function indices. This finding highlights the need to
explore correlation to body weight and, depending on the results, develop weight-specific
reference values coupled with central tendencies of the population, as the current study
and one other study have done. Pariaut and colleagues examined TAPSE in fifty
normal dogs in addition to dogs affected with varying severities of pulmonary
hypertension. Although analyzed slightly differently, they also documented a strong ($r^2 = 0.83$) correlation with body weight and reported weight-specific reference values that are comparable to those reported herein.

The generation of reliable reference values is essential to distinguish healthy from diseased dogs and these cutoffs have the potential to affect important clinical decisions regarding diagnosis, therapy and prognosis. Based on current guidelines for the echocardiographic assessment of the right heart in adult humans, human RV function reference values, including TAPSE, FAC, and S’, are presented as single cutoff values. This methodology is likely problematic, particularly in dogs, given the span of body sizes encountered by the clinician. This problem is also encountered in human pediatric cardiology with growing children and recommendations for the use of body surface area and conversion to Z-scores have been suggested in current guidelines. However, despite these recommendations, more recent studies show that standardization of measurement normalization is still a problem throughout pediatric echocardiographic studies. In the current study, the method ultimately utilized to establish reference values involved regression analysis and demonstrated that allometric (power) scaling provided the best fit for the studied RV function indices. Allometry describes the disproportionate changes in shape, size, or function that are observed when comparing separate isolated features in animals that vary in body size. To quantify this relationship the size of an organ or its function may be expressed as a power function of body mass or weight. Similar to the present study, several investigators have utilized allometric scaling to
normalize indices of cardiac structure or function to body weight in laboratory animals and dogs.52-56

A recent study in people compared the qualitative assessment of RV function to quantitative measures (TAPSE, FAC, S’, and a RV myocardial (Tei) index) using cardiac magnetic resonance as a gold standard and showed a significant increase in accuracy and interobserver agreement with the quantitative measures.1 This study highlights the danger inherent in the qualitative assessment of RV function while also demonstrating the interobserver agreement of the quantitative indices, among which were TAPSE, FAC and S’. Our study also showed that, with the addition of strain and strain rate, the same RV function indices exhibited acceptable inter- and intraobserver agreement in normal dogs. This was also demonstrated in a recent study20 showing that TAPSE exhibits tolerable inter- and intraobserver variability. Furthermore, the current study demonstrated that in conscious healthy dogs TAPSE, FAC, S’ (pulsed wave-derived), strain, and strain rate were reproducible based on the average day-to-day repeatability coefficients of variation that were all <10%. This is comparable to studies in people,14,57 but, to the authors’ knowledge, reproducibility data for the studied RV function indices in dogs have not been reported.

A limitation of the current study is the lack of longitudinal follow-up of the dogs used to define the reference values. Without follow-up, it cannot be certain that the studied dogs did not have subclinical cardiac disease that could affect RV function thereby skewing the reference values. A second limitation to this study is the size of the sample used to generate the reference values, particularly when compared to sample sizes
throughout human medicine. Conversely, when compared to other veterinary studies used to generate reference values, the current study represents one of the largest samples used to generate echocardiographic reference values. Statistically, at least 120 subjects is recommended to generate reference values in order to provide the most reliable cutoffs. To this end, RV function index values that are very close to the recommended cutoffs should be interpreted with caution and always with the clinical picture in mind as well as the potential influence of heart rate for FAC and strain rate. Caution is also warranted when applying the reference values to dogs outside the body weights encountered in the current study (<3.9 kg and >42.3 kg).

To conclude, the current study demonstrated that five indices of RV systolic function (TAPSE, FAC, S’, strain, and strain rate) were feasible and reproducible and had intra- and interobserver variability within clinically acceptable limits. Body weight should be considered when interpreting these RV function indices, as all were shown to correlate with body weight to some degree. Consideration to the dog’s heart rate should be given when interpreting FAC and strain rate values. The current study equips the veterinary cardiologist with body weight-specific reference values for five indices of RV function thereby encouraging the quantitative assessment of RV systolic function in dogs with cardiovascular disease.
Figure 1. Representative measurement of pulmonary velocity time integral (VTI). This measurement was determined by tracing the outer border (dotted white line) of the entire pulsed wave Doppler-derived pulmonary artery flow profile at the level of the pulmonary valve from baseline to baseline. This measurement is directly proportional to stroke volume and with heart rate can be used as a surrogate for right ventricular cardiac output when heart rate is considered.
Figure 2. Representative measurement of tricuspid annular plane systolic excursion (TAPSE). This measurement quantifies maximal longitudinal displacement of the lateral tricuspid valve annulus toward the right ventricular (RV) apex during systole using M-mode echocardiography. Note the cursor (white dotted line) is aligned as parallel as possible to the majority RV free wall. RA, right atrium; RV, right ventricle.
Figure 3. Representative measurement of right ventricular (RV) percent fractional area change (FAC) from a 2D echocardiographic image. Measurements of RV area were obtained by tracing the RV endocardial border (dotted lines) at end-diastole (RVA$_D$) and end-systole (RVA$_S$). FAC is calculated using the formula: FAC = ((RVA$_D$ – RVA$_S$)/RVA$_D$) x 100. RA, right atrium; RV, right ventricle.
Figure 4. Representative measurement of pulsed-wave tissue Doppler imaging (TDI) - derived peak systolic longitudinal myocardial motion velocity at the lateral tricuspid annulus (S’). RA, right atrium; RV, right ventricle.
Figure 5. Representative 2D echocardiographic image of the right heart from the left apical four-chamber view optimized for the right ventricle in which longitudinal segments have been designated by the proprietary software as basilar inferior (basInf; yellow), mid inferior (midInf; light blue) and apical inferior (apInf; green) due to the left ventricular 2Ch algorithm (as there was no right ventricular speckle-tracking echocardiography algorithm at the time of study). The green colored bars with a “V” below the labeled segments indicate that tissue tracking by the software is adequate. RA, right atrium; RV, right ventricle.
Figure 6. Representative snapshot of the workstation output for right ventricular (RV) free wall longitudinal strain. A reference 2D image of the right heart can be seen in the upper left, a color map is shown in the lower left to display the change in strain over one cardiac cycle (red = negative; blue = positive), and the remainder of the snapshot includes 3 regional (basilar = yellow, mid = light blue, apical = green) and global (dotted line) strain curves over time in relation to the ECG (bottom). Only systolic global longitudinal strain of the RV free wall prior to pulmonary valve closure was utilized in this study. “AVC” corresponds to pulmonary valve closure (instead of aortic valve closure [AVC]) determined from a continuous wave Doppler recording of pulmonary outflow timed with the ECG. RA, right atrium; RV, right ventricle.
Figure 7. Representative snapshot of the workstation output for right ventricular (RV) free wall longitudinal strain rate. A reference 2D image of the right heart can again be seen in the upper left, a color map is shown in the lower left to display the change in strain rate over one cardiac cycle (red = negative; blue = positive), and the remainder of the snapshot includes 3 regional (basilar = yellow, mid = light blue, apical = green) and global (dotted line) strain rate curves over time in relation to the ECG (bottom). Only systolic global longitudinal strain rate of the RV free wall was utilized in this study. “AVC”, pulmonary valve closure (instead of aortic valve closure [AVC]); RA, right atrium; RV, right ventricle.
### Tables

**Table 1.** Descriptive statistics for the right ventricular systolic function indices in 80 conscious healthy dogs.

<table>
<thead>
<tr>
<th>RV function index</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>95% CI / IQR</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTI (cm)</td>
<td>14.60</td>
<td>14.41</td>
<td>2.79</td>
<td>13.98 – 15.22</td>
<td>8.65 – 21.32</td>
</tr>
<tr>
<td>TAPSE (mm)</td>
<td>13.75</td>
<td>13.37</td>
<td>3.41</td>
<td>11.40 – 15.53</td>
<td>8.53 – 25.00</td>
</tr>
<tr>
<td>FAC (%)</td>
<td>46.50</td>
<td>46.90</td>
<td>6.57</td>
<td>45.04 – 47.96</td>
<td>32.83 – 62.25</td>
</tr>
<tr>
<td>Strain (\times -1) (%)</td>
<td>28.62</td>
<td>28.58</td>
<td>4.02</td>
<td>27.73 – 29.52</td>
<td>19.95 – 46.41</td>
</tr>
<tr>
<td>Strain Rate (\times -1) (s(^{-1}))</td>
<td>3.29</td>
<td>3.20</td>
<td>0.91</td>
<td>3.09 – 3.49</td>
<td>1.56 – 6.59</td>
</tr>
</tbody>
</table>

RV, right ventricular; VTI, velocity time integral; TAPSE, tricuspid annular plane systolic excursion; FAC, fractional area change; S’, pulsed wave tissue Doppler-derived lateral tricuspid annular longitudinal peak systolic velocity; SD, standard deviation; CI, confidence interval of the mean, IQR, interquartile range.

\(^a\) Data set was not normally-distributed and values represent the interquartile range.
Table 2. Right ventricular function indices repeatability, intra- and inter-observer variability.

<table>
<thead>
<tr>
<th>RV function index</th>
<th>Average coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeatability (n=80)</td>
</tr>
<tr>
<td>VTI</td>
<td>5.9</td>
</tr>
<tr>
<td>TAPSE</td>
<td>4.7</td>
</tr>
<tr>
<td>FAC</td>
<td>4.3</td>
</tr>
<tr>
<td>S’</td>
<td>7.8</td>
</tr>
<tr>
<td>Strain</td>
<td>4.0</td>
</tr>
<tr>
<td>Strain Rate</td>
<td>8.5</td>
</tr>
</tbody>
</table>

See Table 1 for key.
Table 3. Correlation coefficients generated from multiple linear regression analyses demonstrating the relationship between body weight, age, and heart rate compared to the RV systolic function indices.

<table>
<thead>
<tr>
<th>RV function index</th>
<th>Predictors</th>
<th>Zero-order correlation</th>
<th>Part correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAPSE</td>
<td>Body weight</td>
<td>0.858</td>
<td>0.755</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>-0.385</td>
<td>-0.036</td>
<td>0.544</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.149</td>
<td>0.030</td>
<td>0.616</td>
</tr>
<tr>
<td>FAC</td>
<td>Body weight</td>
<td>-0.410</td>
<td>-0.231</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>0.497</td>
<td>0.345</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.059</td>
<td>0.070</td>
<td>0.470</td>
</tr>
<tr>
<td>S’</td>
<td>Body weight</td>
<td>0.550</td>
<td>0.555</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>-0.097</td>
<td>0.126</td>
<td>0.188</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.037</td>
<td>-0.056</td>
<td>0.555</td>
</tr>
<tr>
<td>Strain</td>
<td>Body weight</td>
<td>-0.339</td>
<td>-0.249</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>0.215</td>
<td>0.083</td>
<td>0.441</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.136</td>
<td>-0.099</td>
<td>0.356</td>
</tr>
<tr>
<td>Strain rate</td>
<td>Body weight</td>
<td>-0.610</td>
<td>-0.403</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>0.554</td>
<td>0.308</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-0.020</td>
<td>0.025</td>
<td>0.763</td>
</tr>
</tbody>
</table>

Zero-order correlation coefficients represent the variation between the predictor and the RV function index while ignoring the influence of the other predictors. Part correlations represent the unique variation each predictor shares with the RV function index while controlling (removing) influence of the other predictors. See Table 1 for key.
Table 4. Results of simple linear regression on logarithmically transformed RV function indices and body weight.\(^a\)

<table>
<thead>
<tr>
<th>RV function indices</th>
<th>Log (a)</th>
<th>a</th>
<th>95% Prediction interval for a</th>
<th>b</th>
<th>Std error of Y est</th>
<th>r(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAPSE</td>
<td>0.781</td>
<td>6.039</td>
<td>4.772 – 7.644</td>
<td>0.297</td>
<td>0.0514</td>
<td>0.749</td>
</tr>
<tr>
<td>FAC</td>
<td>1.776</td>
<td>59.70</td>
<td>46.34 – 76.92</td>
<td>-0.097</td>
<td>0.0553</td>
<td>0.216</td>
</tr>
<tr>
<td>S'</td>
<td>0.839</td>
<td>6.898</td>
<td>4.262 – 11.18</td>
<td>0.233</td>
<td>0.1052</td>
<td>0.306</td>
</tr>
<tr>
<td>Strain</td>
<td>1.540</td>
<td>34.67</td>
<td>26.85 – 44.78</td>
<td>-0.075</td>
<td>0.0558</td>
<td>0.140</td>
</tr>
<tr>
<td>Strain rate</td>
<td>0.808</td>
<td>6.427</td>
<td>4.289 – 9.629</td>
<td>-0.263</td>
<td>0.0882</td>
<td>0.440</td>
</tr>
</tbody>
</table>

Linear regression analysis of the logarithmic form of the allometric equation: \(\log (Y) = \log (a) + b \times \log (M)\), where \(a\) is the proportionality constant, \(b\) is the scaling exponent, \(Y\) represents the RV function index, and \(M\) represents body weight, yields the constant \(b\), which is the slope of the regression line and the constant \(a\), which is the antilog \((\log^{-1})\) of the \(y\)-intercept of the regression line. The equation can be rewritten as the allometric equation: \(Y = a \times M^b\).

\(^a\) All correlations were statistically significant (\(P \leq 0.001\)).
### Table 5. Normal RV systolic function indices and 95% prediction intervals for dogs of varying body weights.

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>TAPSE (mm)(^a)</th>
<th>FAC (%)(^b)</th>
<th>S’ (cm/s)(^c)</th>
<th>Strain × -1 (%)(^d)</th>
<th>Strain Rate × -1 (s(^{-1}))(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.6 – 10.6</td>
<td>41.7 – 69.1</td>
<td>5.5 – 14.4</td>
<td>24.7 – 41.2</td>
<td>3.2 – 7.2</td>
</tr>
<tr>
<td>4</td>
<td>7.2 – 11.5</td>
<td>40.5 – 67.2</td>
<td>5.9 – 15.4</td>
<td>24.2 – 40.4</td>
<td>3.0 – 6.7</td>
</tr>
<tr>
<td>5</td>
<td>7.7 – 12.3</td>
<td>39.6 – 65.8</td>
<td>6.2 – 16.3</td>
<td>23.8 – 39.7</td>
<td>2.8 – 6.3</td>
</tr>
<tr>
<td>7</td>
<td>8.5 – 13.6</td>
<td>38.4 – 63.7</td>
<td>6.7 – 17.6</td>
<td>23.2 – 38.7</td>
<td>2.6 – 5.8</td>
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<td>7.1 – 18.7</td>
<td>22.8 – 38.0</td>
<td>2.4 – 5.4</td>
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<td>36.4 – 60.5</td>
<td>7.6 – 19.9</td>
<td>22.3 – 37.2</td>
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<td>2.1 – 4.7</td>
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<td>8.6 – 22.5</td>
<td>21.4 – 35.8</td>
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<td>21.1 – 35.2</td>
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<td>9.4 – 27.4</td>
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<td>32.8 – 54.5</td>
<td>9.8 – 25.6</td>
<td>20.6 – 34.3</td>
<td>1.7 – 3.8</td>
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<td>14.3 – 22.8</td>
<td>32.4 – 53.8</td>
<td>10.1 – 26.4</td>
<td>20.4 – 34.0</td>
<td>1.6 – 3.6</td>
</tr>
<tr>
<td>45</td>
<td>14.8 – 23.7</td>
<td>32.0 – 53.2</td>
<td>10.3 – 27.1</td>
<td>20.2 – 33.7</td>
<td>1.6 – 3.5</td>
</tr>
</tbody>
</table>

\(^a\) allometric equation with 95% prediction intervals: Y = 4.777 to 7.640 \times M\(^{0.297}\)

\(^b\) allometric equation with 95% prediction intervals: Y = 46.34 to 76.92 \times M\(^{-0.097}\)

\(^c\) allometric equation with 95% prediction intervals: Y = 4.812 to 11.66 \times M\(^{0.233}\)

\(^d\) allometric equation with 95% prediction intervals: Y = 26.851 to 44.776 \times M\(^{-0.075}\)

\(^e\) allometric equation with 95% prediction intervals: Y = 4.029 to 8.543 \times M\(^{-0.236}\)

See Table 1 for key.
Footnotes

a Vivid 7 Dimension with EchoPac software package, version BT09, GE Medical Systems, Waukesha, WI, USA.

b EchoPAC 2D Strain software, Q-Analysis (strain module), version 6.1, GE Medical Systems, Waukesha, WI, USA.

c http://www.randomizer.org/

d IBM SPSS Statistics, version 21, IBM Corp, Armonk, NY, USA.

e MedCalc, version 12.7.4, MedCalc Software, Ostend, Belgium.
References


Chapter 3:

Echocardiographic Assessment of Right Ventricular Systolic Function in Conscious Healthy Dogs Following a Single Dose of Pimobendan Versus Atenolol: A Prospective, Blinded, Randomized, Crossover Study

Abstract

Objective: To determine if pulmonary velocity time integral (VTI), tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), pulsed wave tissue Doppler-derived systolic myocardial velocity of the lateral tricuspid annulus (S’), and speckle-tracking echocardiography-derived systolic global longitudinal right ventricular (RV) free wall strain and strain rate could identify anticipated changes in RV systolic function following an oral dose of pimobendan and atenolol as compared to baseline.

Animals: 80 conscious healthy dogs.

Methods: All dogs underwent 4 echocardiograms – twice for baseline, once 3-hours post-pimobendan (0.25 mg/kg) and once 3-hours post-atenolol (1 mg/kg). Wash-out period between drugs was 3 to 20 days. Post-drug RV function indices were compared to their respective baseline. Mixed model analysis was performed comparing percent changes from baseline among treatments (pimobendan or atenolol) and other covariates of interest (heart rate, age, weight, gender, drug sequence, and time period).

Results: All RV function indices showed a significant (P < 0.0001) increase and decrease from baseline following pimobendan and atenolol, respectively. Treatment had a significant (P < 0.0001) effect on percent change from baseline for all RV function
indices. Other covariates were not significant, except heart rate which had a significant (P = 0.02) effect on pulmonary VTI. Post-atenolol, a significantly greater proportion of dogs exceeded the day-to-day coefficient of variation for FAC and S’ compared to TAPSE (P ≤ 0.007). A significantly greater proportion of dogs had an FAC outside the reference interval compared to TAPSE and strain post-pimobendan (P ≤ 0.005) and TAPSE and S’ post-atenolol (P ≤ 0.009). S’ and strain rate exhibited the greatest percent change from baseline post-pimobendan and atenolol (P < 0.0001).

Conclusions: The RV functional indices evaluated consistently tracked anticipated changes in RV systolic function following pimobendan and atenolol in healthy dogs, thus warranting further study in dogs with cardiovascular disease.

Introduction

For decades, the right ventricle has been considered less relevant to cardiac function and, until recently, was ignored. The importance of the quantitative assessment of right ventricular (RV) function with regard to clinical status and outcome is increasingly apparent in people affected with both cardiac and non-cardiac diseases.\(^1\) There is compelling evidence that RV systolic function status provides prognostic data and aids in clinical decision-making in people with right heart-specific diseases,\(^2\) and left heart-specific diseases, including mitral and aortic valve disease\(^3-5\) and idiopathic dilated cardiomyopathy,\(^6-11\) often independent of pulmonary hypertension status. As dogs are affected by many similar diseases, a study of the quantitative assessment of RV function in dogs is warranted.
Echocardiography is the most practical method for assessment of RV structure and function, as it is noninvasive, readily available, relatively inexpensive, and does not require general anesthesia. However, the echocardiographic assessment of RV function is often challenging due to its load-dependence, coarse trabeculae, and its subdivided anatomy that is less amenable to geometric shape assumptions.\textsuperscript{12} Despite these challenges, and in contrast to the veterinarian, physicians utilize a number of echocardiographic indices of RV function that have been validated against a catheterization- or cardiac magnetic resonance (CMR)-derived gold standard.\textsuperscript{13-17}

The velocity time integral (VTI) of the pulmonary outflow signal is directly proportional to stroke volume and can be considered a surrogate for cardiac output of the right ventricle when multiplied by heart rate, as this variable is an integration over time of the flow out of the right heart. When multiplied by the cross-sectional area of the pulmonary valve annulus, stroke volume can be derived.\textsuperscript{18} Tricuspid annular plane systolic excursion (TAPSE) is one of the simplest methods to assess RV longitudinal function. Measurement of TAPSE involves determination of the apical displacement of the tricuspid valve annulus during systole from an M-mode recording. A recent study\textsuperscript{19} showed that TAPSE is decreased in dogs with varying severities of pulmonary hypertension (PH) and below the reference interval in most dogs with severe PH. Percent fractional area change (FAC) of the right ventricle represents a 2-dimensional (2D) surrogate of RV ejection fraction (EF) from an apical imaging plane and is calculated from planimetered measurements of the RV chamber at end-diastole and end-systole. The end-systolic area is subtracted from the end-diastolic area with the difference then
divided by the end-diastolic area. In people, TAPSE and FAC are considered practical and are routinely performed in patients with cardiac disease. Tissue Doppler imaging (TDI)-derived systolic myocardial velocity of the lateral tricuspid annulus (S’) represents a region-specific index of RV longitudinal systolic function. Pulsed wave TDI-derived RV myocardial velocity is appealing because it is easy to obtain and strongly correlates with an invasive gold standard in the dog. Color TDI-derived RV myocardial velocities have been shown to be repeatable and reproducible, and predictive of varying severities of PH in the dog. Color TDI-derived RV systolic velocities may also be of value in the early prediction of dilated cardiomyopathy in the dog. Speckle-tracking echocardiography (STE)-derived strain and strain rate are relatively new indices of myocardial function and are based on quantifying tissue deformation (strain) or tissue deformation over time (strain rate) globally or in a region-specific manner. A large advantage of STE-derived indices is their angle-independence, which is seemingly well-suited for the right ventricle given its complex geometry.

The aforementioned echocardiographic indices of RV systolic function may be applicable in the identification and quantification of RV dysfunction in the dog, but studies attempting to validate these indices in addition to reference values (see Chapter 2) are needed before they can become clinically useful in dogs with diverse cardiopulmonary diseases. Therefore, the current study sought to study 5 echocardiographic indices of RV systolic function comprehensively in the conscious healthy dog under 3 contractile states (baseline, increased, and decreased). Specifically, the objective of this study was to determine if TAPSE, FAC, S’, global RV free wall
STE-derived strain and strain rate could track changes in RV systolic function following a single oral dose of pimobendan and atenolol in dogs compared to baseline. We hypothesized that the proposed echocardiographic indices could detect changes in RV systolic function following the administration of pimobendan (increase in systolic function) and atenolol (decrease in systolic function) in conscious healthy dogs.

**Materials and Methods**

All procedures in this study were approved by the Institutional Animal Care and Use Committee and the Veterinary Medical Center Clinical Research and Teaching Advisory Committee at The Ohio State University. Written consent authorizing participation of dogs in the study was obtained from all dog owners.

**Study Subjects**

The same study subjects presented in Chapter 2 were utilized for the current study and enrollment criteria were identical.

**Study Design**

This was a prospective, single-blinded, randomized, crossover study. Immediately following a baseline echocardiographic study, each dog was administered a single dose of either the pimobendan (\(\sim 0.25 \text{ mg/kg}\)) or atenolol (\(\sim 1 \text{ mg/kg}\)) by mouth. The order of drug administration was randomized and the investigators were blinded to the drug’s identity. Three hours post-pill, all dogs had the same echocardiographic study performed
to evaluate the effect of the given drug on RV systolic function. In the dog, peak blood concentration of both pimobendan\textsuperscript{24} and atenolol\textsuperscript{25} have been shown to occur within approximately 3 hours following oral administration. Within a 3 week time period and following a minimum 72 hour washout period, each dog had a second baseline and post-drug echocardiographic study using the alternate drug. Thus, each dog underwent 4 echocardiographic studies that were performed by the same sonographer (LCV) – twice for baseline, following pimobendan, and following atenolol.

\textit{Echocardiographic Examination}

Echocardiographic studies were performed exactly as described in Chapter 2.

\textit{Statistical Analysis}

All statistical analyses were performed using commercial software.\textsuperscript{d,e,f} Normality testing for continuous data consisted of visual inspection of probability plots and the D’Agostino-Pearson test. A value of \( P < 0.05 \) was considered statistically significant.

For each echocardiographic index of RV systolic function following each drug, a paired t-test (or Wilcoxon signed-rank test) was used to compare baseline to its respective post-drug echocardiogram.

For each RV function index, a linear mixed-effects regression model was used to compare percent change in RV function from the same-day baseline. The model included random effects (dog) and fixed effects (treatment [pimobendan or atenolol], heart rate, body weight [in kg], gender [male or female], age [in months], drug sequence, and time
period). The heart rate value was determined as the average heart rate of each of the 5 representative cardiac cycles used to determine the RV function index value post-drug. For all linear regression models, assumptions of linearity, homoscedasticity, and normality of the residuals were evaluated by inspection of the standardized residual plots and probability plots.

The pulmonary VTI was used to confirm that an increase in RV cardiac output occurred with the administration of pimobendan and a reduction with atenolol, but was not considered a useful variable of RV function by itself given higher variability than the other indices (see Chapter 2). As such, further analysis was continued for TAPSE, FAC, S’, global RV free wall STE-derived strain and strain rate, but not for pulmonary VTI.

In order to compare the RV function indices post-pimobendan and post-atenolol, a chi-squared test was performed on the proportion of dogs whose RV function index did or did not exceed the average repeatability coefficient of variation (i.e., the day-to-day variability) specific to each RV function index (see Chapter 2, Table 2). A similar analysis was performed on the proportion of dogs whose RV function index was or was not outside the body weight-specific reference interval specific to each RV function index (see Chapter 2, Table 5). If the chi-squared test was statistically significant, post-hoc comparison of RV function indices consisted of multiple z-tests comparing 2 proportions while adjusting the P-value based on the Holm-Bonferroni (sequentially rejective) method for the number of comparisons.

A Friedman’s test was performed as an additional method to compare the RV function indices’ percent change in RV function from baseline post-pimobendan and
post-atenolol. If the test was statistically significant, post-hoc comparisons of the RV function indices consisted of multiple Wilcoxon signed rank tests while adjusting the P-value based on the Holm-Bonferroni (sequentially rejective) method for the number of comparisons.

**Results**

*Study Subjects*

The study sample is exactly as presented in Chapter 2.

*RV Function Indices Following Pimobendan and Atenolol*

Right ventricular systolic function indices before and after pimobendan and atenolol are summarized in Table 6 and the percent change from respective baseline following each drug is displayed graphically in Figure 8. For each RV function index following each drug, a statistically significant difference (all P < 0.0001) was encountered when each baseline study was compared to its same-day post-drug echocardiogram (Table 6).

Mean percent change in RV systolic function from baseline after pimobendan and atenolol was significantly different (i.e., treatment effect was significant) for each RV systolic function index (all P < 0.0001). With the exception of the effect of heart rate on pulmonary VTI (P = 0.02), none of the other fixed effects (gender, body weight, age, heart rate, drug sequence, or time period) demonstrated a significant effect on the model for any of the RV function indices.
The RV function indices detected changes in RV function exceeding the average repeatability coefficient of variation (day-to-day variability) post-pimobendan or atenolol in at least 59 of 80 dogs (Table 7). Chi-squared analysis comparing the proportion of dogs whose RV function index post-pimobendan exceeded the repeatability coefficient of variation failed to reach statistical significance (P = 0.069). FAC and S’ had a significantly higher proportion of dogs whose RV function index post-atenolol exceeded the repeatability coefficient of variation compared to TAPSE (both P ≤ 0.007).

Compared to detecting changes beyond the repeatability coefficient of variation, all of the RV function indices were less sensitive to changes falling outside the body weight-specific reference intervals (see Chapter 2, Table 5) post-drug (Table 8). FAC had a significantly higher proportion of dogs that were above the reference interval post-pimobendan compared to TAPSE and strain (both P ≤ 0.005). Following atenolol, FAC had a significantly higher proportion of dogs who were below the reference interval compared to TAPSE and S’ (both P = 0.009).

Comparing the RV function indices based on the percent increase from baseline post-pimobendan (Figure 9), both S’ and strain rate detected the largest increase in RV function from baseline and were both significantly (all P < 0.0001) greater than the other indices but not significantly different from each other. FAC detected a significantly larger increase in RV function from baseline compared to strain and TAPSE (both P < 0.0001). Following atenolol, S’ and strain rate detected the largest decrease in RV function from baseline and were both significantly (all P ≤ 0.002) greater than FAC, strain, and TAPSE.
but were not significantly different from each other (Figure 10). FAC detected a significantly larger decrease in RV function compared to TAPSE ($P < 0.0001$).

**Discussion**

The results presented confirmed our hypothesis that the studied echocardiographic indices of RV systolic function can track changes in RV systolic function following the oral administration of inotropic drugs at clinically-relevant doses in conscious healthy dogs. With the exception of the effect of heart rate on pulmonary VTI, the other RV function indices’ percent change in RV function post-drug were not statistically affected by heart rate, body weight, gender, age, drug sequence, or time period. Only the treatment effect on the percent change in RV function post-drug was significant for all RV function indices in the sample studied. All of the RV function indices were able to detect changes beyond the day-to-day variability in the majority of dogs; as such, these indices should detect clinically relevant changes in RV systolic function.

In veterinary medicine, studies designed to validate echocardiographic indices of cardiac function have traditionally compared a cardiac function index to an invasive gold standard, an inherent advantage of that type of study design. However, these studies commonly involve a small number of dogs of similar body type or breed e.g., purpose-bred research beagles or hounds that are subject to the negative inotropic effects and risks of general anesthesia thus limiting clinical extrapolation of these results. These types of studies may also warrant caution when extrapolating results to the general population due to homogeneity in the study groups. The study design utilized herein did not require the
use of general anesthesia and included a large, diverse study sample consisting of dogs of various size, breed, and age. Therefore, we consider our study design clinically relevant and an acceptable alternative to traditional methods, despite the lack of an invasive gold standard.

With the exception of S’, very few validation studies on the echocardiographic assessment of RV systolic function have been performed in the dog. Hori et al. demonstrated that pulsed wave TDI-derived S’ strongly correlated (r = 0.93) with RV contractility (+dP/dt) over a range of inotropic states in seven anesthetized beagles. Interestingly, in the study by Hori et al., S’ was not significantly decreased compared to baseline following the intravenous administration of the negative inotrope esmolol (low or high dose), which is in contrast to the current study where S’ was significantly decreased from baseline following oral administration of atenolol. The inability of Hori et al. to measure a difference may be explained by depression of RV systolic function secondary to general anesthesia in these dogs prior to the administration of the esmolol, in addition to a lack of statistical power given the small number of dogs. S’ has also been shown to correlate well with radionuclide ventriculography in people with a variety of right heart diseases, and with CMR-derived RV ejection fraction (EF) in people with congenital heart disease and arrhythmogenic RV cardiomyopathy. Advantages of pulsed wave TDI-derived S’ include that it is quick, easy-to-perform, and less geometry-dependent than other variables. It is limited by its angle-dependence, region specificity, and, together with TAPSE, it has been shown to be less accurate in the setting of severe tricuspid regurgitation in people.
TAPSE has been validated in healthy people\textsuperscript{30} and has been shown to correlate well with radionuclide-\textsuperscript{31} and CMR-derived RV EF\textsuperscript{32} in people with RV dysfunction. Similar to S', TAPSE is a quick, simple, and less geometry-dependent index of RV function. It is, however, another angle-dependent, region-specific index that may be adversely affected by translational motion, which may particularly be a problem in dogs.

Percent FAC has demonstrated to correlate best to CMR-derived RV EF when compared to other 2D echocardiographic indices of RV systolic function in people, including TAPSE.\textsuperscript{33} Unlike the other RV function indices studied in the current report, FAC is not solely an index of RV longitudinal function and incorporates an additional plane to assess RV function. Despite its ease to perform and less acquisition angle-dependence, it may be less-favorable in patients with poor image quality because of the necessity for accurate RV endocardial border detection.

Right ventricular free wall STE-derived longitudinal strain and strain rate were recently demonstrated to correlate well with CMR-derived RV EF, mean pulmonary artery pressure, and pulmonary vascular resistance in people affected with PH.\textsuperscript{34} Despite their post-processing and more time-consuming nature, strain and strain rate are attractive indices primarily due to angle-independence and both global and regional function assessment capabilities. Thus, each of the studied RV function indices have also been validated in people and each possess their own inherent advantages and disadvantages.

The current study was designed to determine if 5 echocardiographic indices of RV systolic function could detect changes post-pimobendan and atenolol compared to baseline in healthy dogs. It was not necessarily designed to compare the 5 RV systolic
function indices to each other as none are a recognized gold standard. Several post hoc comparisons attempting to determine differences in the RV function indices’ ability to track changes in the inotropic state were performed. We view the comparison of the RV function indices involving the proportion of dogs whose RV function index surpassed the day-to-day coefficient of variation post-drug as most reflective of and relevant to clinical practice. The comparison of RV function indices involving the proportion of dogs whose RV function index surpassed the day-to-day coefficient of variation post-atenolol may be the most clinically relevant, as most diseases that affect the right ventricle are likely to cause a decrease in function. Based on our results, FAC and S’ may be better suited to detect decreases in RV function beyond day-to-day variability than TAPSE in diseased dogs; but studies designed to specifically test this hypothesis would be required.

Similarly, the comparison of the RV function indices based on the proportion of the dogs whose function index was beyond the reference interval post-drug warrants caution, as this was assessed in a sample of healthy dogs. The comparison of the RV function indices’ percent change in RV function post-drug does not necessarily mean that the index with the greatest percent change is superior to the others at tracking the inotropic state. Results of these comparisons may indicate that certain RV function indices such as S’ and strain rate are more sensitive at tracking the inotropic state but the variability of these indices must also be kept in mind. Ultimately, it is our hope that the current study and the post-hoc comparisons performed stimulate further studies assessing and comparing RV function indices in dogs affected with various cardiopulmonary diseases.
To conclude, the current study showed that 5 echocardiographic indices of RV systolic function, each with their own inherent advantages and disadvantages, were consistently able to detect changes in RV systolic function following induced positive and negative inotropic states when compared to baseline in conscious healthy dogs. Further studies are warranted to determine the value of these RV function indices in dogs with cardiovascular or respiratory disease.
**Figures**

**Figure 8.** Mean ± SD percent (%) change in right ventricular (RV) systolic function from baseline following pimobendan (black line) and atenolol (gray line) as quantified by pulmonary velocity time integral (VTI), tricuspid annular plane systolic excursion (TAPSE), RV percent fractional area change (FAC), pulsed wave tissue Doppler-derived lateral tricuspid annular longitudinal peak systolic velocity (S’), RV free wall global longitudinal strain (“Strain”) and RV free wall global longitudinal strain rate (“Strain rate”). Percent change in RV systolic function from baseline post-atenolol was significantly different compared to post-pimobendan for each RV systolic function index (all P < 0.0001). Note both the degree of change (% change from baseline) and the variation of the change (SD error bars) following pimobendan and atenolol for each RV systolic function index.
Figure 9. Median ± interquartile range for percent change (increase) in right ventricular (RV) function as quantified by tricuspid annular plane systolic excursion (TAPSE), RV percent fractional area change (FAC), pulsed wave tissue Doppler-derived lateral tricuspid annular longitudinal peak systolic velocity (S’), RV free wall global strain (“Strain”) and RV free wall global strain rate (“Strain Rate”) post-pimobendan. A Friedman’s test showed the RV function indices were statistically different (P < 0.0001).

*aPercent increase in RV function from baseline was significantly greater than FAC, strain, and TAPSE (all P < 0.0001). bPercent increase in RV function from baseline was significantly greater than strain and TAPSE (both P < 0.0001).
Figure 10. Median ± interquartile range for percent change (decrease) in RV function as quantified by TAPSE, FAC, S’, Strain, and Strain rate post-atenolol. A Friedman’s test showed the RV function indices were statistically different (P < 0.0001). aPercent decrease in RV function from baseline was significantly greater than FAC, strain, and TAPSE (all P ≤ 0.002). bPercent decrease in RV function from baseline was significantly greater than TAPSE (P < 0.0001). See Figure 9 for the key.
### Table 6. Echocardiographic right ventricular systolic function indices (mean ± SD) at baseline and 3-hours post-pimobendan and atenolol.

<table>
<thead>
<tr>
<th>RV function index</th>
<th>Pimobendan</th>
<th>Atenolol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>3-hours post</td>
</tr>
<tr>
<td>VTI (cm)</td>
<td>14.76 ± 2.93</td>
<td>16.84 ± 3.16</td>
</tr>
<tr>
<td>TAPSE (mm)</td>
<td>13.73 ± 3.40</td>
<td>15.44 ± 3.80</td>
</tr>
<tr>
<td>FAC (%)</td>
<td>46.68 ± 6.75</td>
<td>56.01 ± 7.84</td>
</tr>
<tr>
<td>S' (cm/s)</td>
<td>13.38 ± 3.82</td>
<td>17.63 ± 5.51</td>
</tr>
<tr>
<td>Strain × -1 (%)</td>
<td>28.53 ± 4.03</td>
<td>32.28 ± 4.53</td>
</tr>
<tr>
<td>Strain Rate × -1 (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.27 ± 0.94</td>
<td>4.27 ± 1.38</td>
</tr>
</tbody>
</table>

RV, right ventricular; VTI, velocity time integral; TAPSE, tricuspid annular plane systolic excursion; FAC, fractional area change; S’, pulsed wave tissue Doppler-derived lateral tricuspid annular longitudinal peak systolic velocity.

<sup>a</sup> Each RV function index demonstrated a significant (all P < 0.0001) increase compared to its respective baseline.

<sup>b</sup> Each RV function index demonstrated a significant (all P < 0.0001) decrease compared to its respective baseline.

<sup>c</sup> For each RV function index, mean percent (%) change in RV systolic function post-drug had a significant (all P < 0.0001) effect based on the mixed-model analysis.
Table 7. Comparison of right ventricular function indices using the proportion of dogs whose right ventricular function post-pimobendan and atenolol exceeded the repeatability coefficient of variation (i.e., the day-to-day variability) for the specific right ventricular function index.

<table>
<thead>
<tr>
<th>RV function index</th>
<th>Proportion of dogs whose RV function index post-pimobendan exceeded the repeatability CV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Proportion of dogs whose RV function index post-atenolol exceeded the repeatability CV&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAPSE</td>
<td>64/80</td>
<td>59/80</td>
</tr>
<tr>
<td>FAC</td>
<td>75/80</td>
<td>76/80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S’</td>
<td>68/80</td>
<td>73/80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Strain</td>
<td>73/80</td>
<td>72/80</td>
</tr>
<tr>
<td>Strain Rate</td>
<td>71/80</td>
<td>68/80</td>
</tr>
</tbody>
</table>

CV, coefficient of variation. See Table 6 for reminder of the key.

<sup>a</sup> Chi-squared analysis comparing the RV function indices post-pimobendan did not reach statistical significance (P = 0.069).

<sup>b</sup> When comparing the RV function indices post-atenolol, FAC and S’ were significantly different from TAPSE (both P ≤ 0.007).
Table 8. Comparison of right ventricular function indices using the proportion of dogs whose right ventricular function index post-pimobendan and atenolol were outside the body weight-specific reference interval.

<table>
<thead>
<tr>
<th>RV function index</th>
<th>Proportion of dogs whose RV function index post-pimobendan was above the RI</th>
<th>Proportion of dogs whose RV function index post-atenolol was below the RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAPSE</td>
<td>8/80</td>
<td>15/80</td>
</tr>
<tr>
<td>FAC</td>
<td>27/80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31/80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S’</td>
<td>20/80</td>
<td>15/80</td>
</tr>
<tr>
<td>Strain</td>
<td>11/80</td>
<td>26/80</td>
</tr>
<tr>
<td>Strain Rate</td>
<td>20/80</td>
<td>26/80</td>
</tr>
</tbody>
</table>

RI, reference interval. See Table 6 for reminder of the key.

<sup>a</sup> When comparing the RV function indices post-pimobendan, FAC was significantly different from TAPSE and strain (both \( P \leq 0.005 \)).

<sup>b</sup> When comparing the RV function indices post-atenolol, FAC was significantly different from TAPSE and S’ (both \( P = 0.009 \)).
Footnotes

a Vetmedin, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA

b Atenolol, Ranbaxy Pharmaceuticals, Princeton, NJ, USA

c http://www.randomizer.org/

d GraphPad Prism, version 5.04, GraphPad Software, Inc., San Diego, CA, USA

e SAS, version 9.2, SAS Institute Inc., Cary, NC, USA

f MedCalc, version 12.7.4, MedCalc Software, Ostend, Belgium.
References


21. Chetboul V, Sampedrano CC, Gouni V, et al. Quantitative assessment of regional right ventricular myocardial velocities in awake dogs by Doppler tissue imaging:


Chapter 4:
Concluding Remarks

The goal of this thesis was to comprehensively study several echocardiographic indices of right ventricular (RV) systolic function in conscious healthy dogs in order to generate repeatability data, reliable reference values, and validate these indices in a clinically relevant manner. We felt this was a necessary step prior to wide-spread clinical use of the studied echocardiographic indices of RV systolic function. It is also our hope that the work presented stimulates further study of RV function in dogs with cardiovascular disease. The work presented herein generally supported our hypotheses that: 1) the studied echocardiographic indices of RV systolic function are feasible with acceptable repeatability, interobserver variability, and intraobserver variability in a large sample of conscious healthy dogs; and 2) the RV function indices could detect changes in RV systolic function following a single oral dose of pimobendan (increase in systolic function) and atenolol (decrease in systolic function) in conscious healthy dogs.

To summarize, the results presented in chapter 2 demonstrated that, aside from the interobserver variability data for pulmonary velocity time integral (VTI), the studied RV systolic function indices were feasible with adequate repeatability and intra- and interobserver variability. Thus, all of the RV function indices studied, with the exception of pulmonary VTI, were deemed suitable for RV function assessment. All of the indices
were found to correlate to at least some degree with body weight. Also, global RV free wall strain rate (“strain rate”) and fractional area change (FAC) exhibited a moderate positive correlation with heart rate. Body weight-specific reference values for the 5 RV function indices were also presented. The results presented in chapter 3 revealed that all of the studied RV systolic function indices showed an expected increase and decrease from baseline following oral administration of pimobendan and atenolol, respectively. Aside from the drug effect and heart rate for pulmonary VTI, heart rate, age, weight, gender, drug sequence, and time period did not show a significant effect on percent change in RV function post-drug for any of the RV function indices. Post-atenolol, FAC and pulsed wave tissue Doppler-derived systolic myocardial velocity of the lateral tricuspid annulus (S’) had a greater proportion of dogs whose RV function index exceeded the day-to-day coefficient of variation compared to tricuspid annular plane systolic excursion (TAPSE). After drug administration, a greater proportion of dogs had an FAC outside the reference interval as compared to TAPSE and global RV free wall strain (“strain”) post-pimobendan, as well as to TAPSE and S’ post-atenolol. S’ and strain rate exhibited the greatest percent change from baseline post-pimobendan and atenolol.

Taken together, the results presented in chapters 2 and 3 demonstrate that TAPSE, FAC, S’, strain and strain rate are feasible, repeatable and reliable indices of RV systolic function in conscious healthy dogs. We feel the results presented within this thesis support the use of these echocardiographic indices of RV systolic function and should permit further echocardiographic study of RV systolic function in dogs affected with
cardiovascular disease. Potential future directions to consider include the application of these echocardiographic indices to common canine cardiovascular diseases that affect the right ventricle such as congenital pulmonary valve stenosis, tricuspid valve malformation, ARVC in the Boxer dog, or pulmonary hypertension, in order to determine if they play a role in the diagnosis, treatment or prognosis of these diseases. Furthermore, determining the value of the echocardiographic assessment of RV function in common left heart diseases such as canine degenerative valve disease and dilated cardiomyopathy also represents another potential future direction of research based on the results of this thesis, coupled with the now recognized benefit of this information in the current human literature. Although this study did not assess RV function in other veterinary species, it is tempting to also consider the potential value of these indices of RV function in other veterinary species commonly affected with cardiovascular disease such as the cat or horse. Thus, future studies on the echocardiographic assessment of RV function in healthy cats or horses may also be a future direction to consider.
References

Chapter 1 References:


34. Rudski LG, Lai WW, Afilalo J, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a


Chapter 2 References:


Chapter 3 References:


